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Pacific Islands Fisheries Science Center
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# Chemoreception in Loggerhead Sea Turtles: An Assessment of the Feasibility of Using Chemical Deterrents to Prevent Sea Turtle Interactions with Longline Fishing Gear

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## ABSTRACT

The mechanisms by which sea turtles are attracted to and become hooked and entangled in commercial fishing gear are not well understood. Identification of sensory attractants and repellants may prove useful in developing gear and bait modifications to reduce sea turtle bycatch in commercial fisheries. We conducted experiments to investigate the ability of loggerhead turtles (*Caretta caretta*, Linnaeus 1758) to use chemical and flow cues to successfully locate squid bait and also tested to see if chemical modification of squid bait would reduce the turtles' ability and/or willingness to track and locate bait. Captive-reared juvenile loggerhead turtles were placed in a seawater-filled flume tank with a current of 3–5 $cm \cdot sec^{-1}$. A nylon bag containing either nylon (control), squid, or squid that had been marinated in 2-phenylethanol or shark-derived compounds was placed in the current upstream from the turtle. Trials were conducted in darkness, and the behavior of turtles was monitored and recorded using an IR-sensitive video surveillance system. The presence of squid bait in the tank elicited feeding and searching behavior; however, turtles showed limited ability to locate squid bait in the absence of visual cues. Only 25–33% of turtles located and ate the squid bait during the 10-minute trial period. These results indicate that visual cues are important for foraging success in loggerhead turtles, and chemoreception likely plays a secondary role. Treatment of squid with 2-phenylethanol or shark-derived compounds did not prevent turtles from eating squid bait. There was no significant difference in the number of turtles that located and ate bait between control, squid, and chemically modified squid trials. An effective chemical deterrent for sea turtles has yet to be identified.



Keywords: behavior, bycatch, fisheries, olfaction, sea turtle

## INTRODUCTION

The incidental capture of sea turtles in commercial longline fishing gear is an issue of growing concern for fishers, fisheries management agencies, and environmental groups. Bycatch of sea turtles in longline gear designed to capture pelagic fish species is a source of mortality for endangered leatherback turtles (*Dermochelys coriacea*, Vandelli 1761), loggerhead turtles (*Caretta caretta*, Linnaeus 1758), green turtles (*Chelonia mydas*), and olive ridley turtles (*Lepidochelys olivacea*, Eschscholtz 1829) in the North Pacific Ocean (Balazs and Pooley, 1994). To help speed the recovery of sea turtle populations, the National Marine Fisheries Service (NMFS; NOAA Fisheries) has enacted numerous mitigation measures to reduce or prevent the incidental capture of sea turtles by U.S. longline fleets in all oceans (www.nmfs.noaa.gov/pr/PR3/regulations.html). Regulations currently required in all U.S. longline fishing operations, such as use of large circle hooks and fish bait, may not be viable options in all foreign fisheries, which account for a much larger fishing effort than the U.S. longline fleets (Lewison et al., 2004.) Accordingly, alternative methods to minimize or prevent sea turtle bycatch in longline gear are being investigated.

Sea turtles and pelagic fishes are evolutionarily distinct groups of animals with differences in sensory biology that may influence the ways in which they interact with fishing gear. The factors that attract sea turtles and target fish species to longline gear and bait are not well understood, but numerous sensory cues may be involved. In 2000, a multidisciplinary interagency collaborative effort was initiated by NOAA Fisheries scientists to investigate the visual, auditory, and chemosensory abilities of sea turtles and pelagic fishes to identify differences in sensory abilities that may be exploited to develop gear and bait attractive to fish but undetectable or unattractive to sea turtles. This paper presents results from a series of studies designed and conducted to assess the chemosensory abilities of loggerhead turtles and explores the feasibility of using chemical deterrents to prevent sea turtles from interacting with longline fishing gear.

The term "chemoreception" refers to an organism's ability to detect and differentiate chemical cues in its environment by taste (gustation) or smell (olfaction). Chemical cues may be used for prey detection and location (Zimmer and Butman, 2000), orientation during long distance migrations (Atema et al., 2002; Doving and Stabell, 2003), or intraspecific communication related to reproduction and predator avoidance (Weldon, 1990; Hara, 1993; Wisenden, 2003 and references therein). The role of chemoreception in the ecology of marine invertebrates and fishes has been well-studied, but relatively little information is available on the ecological importance of chemoreception for marine reptiles.

We were primarily interested in determining the role of chemoreception in the foraging and avoidance behaviors of loggerhead turtles. Although sea turtles are generally considered to be visual predators, other sensory cues (tactile, flow, chemical) may also

contribute to foraging success. There is compelling evidence that chemoreception is an important factor in food recognition by post-hatchling and juvenile sea turtles (Grassman and Owens, 1982; Constantino and Salmon, 2003), but the ability of sea turtles to use chemical cues to effectively track and locate prey has not been studied. If sea turtles use chemoreception to detect and find food sources in their aquatic environment, then chemicals emanating from squid and mackerel bait may play a role in attracting sea turtles to longline fishing gear. Chemical modifications that make bait less appealing or more difficult for sea turtles to detect may help deter sea turtles from interacting with fishing gear.

The ability of aquatic organisms to use chemical cues to locate prey can be affected by numerous factors. Results from studies of chemical tracking behavior in marine invertebrates and fishes show that flow patterns, in particular, play a critical role in the successful location of prey in a dynamic and complex environment. Orientation into flow (rheotaxis) is a common component of searching strategies, as the most likely source of an odor would be upstream (Hodgson and Mathewson, 1978; Weissburg and Zimmer-Faust, 1994; Zimmer-Faust et al., 1995; Vickers, 2000; Carton and Montgomery, 2003; Kanter and Coombs, 2003). We designed a flume tank in which squid bait was presented to loggerhead turtles under unidirectional flow conditions to assess the ability of loggerhead turtles to use chemical cues in combination with flow cues to locate a food source in the absence of visual cues. These experiments were designed to gauge the relative importance of chemoreception for successful prey location. The flume tank was also used to investigate behavioral responses of loggerhead turtles to bait that had been chemically modified. We presented loggerheads with squid that had been treated with a chemical odor-masking agent, 2-phenylethanol. Loggerhead turtles are capable of detecting 2-phenylethanol, although in previous studies they showed no sign of attraction to this chemical and are unlikely to identify it with a food source at first exposure (Manton et al. 1972; Southwood et al., 2005). We also presented loggerhead turtles with squid that had been treated with skin secretions of tiger sharks (*Galeocerdo cuvier*, Lesueur 1822), a known predator of sea turtles. Several species of reptile display avoidance and defensive behavior when presented with skin extracts and rinses from predators (Dial, 1990; Weldon, 1990), and we were interested in assessing the potential for using predator-derived compounds to elicit avoidance behavior in sea turtles. We hypothesized that loggerhead turtles presented with chemically modified squid would exhibit a lower rate of success in locating bait compared with turtles presented with untreated squid.

## MATERIALS AND METHODS

### Test Animals

All experiments were conducted with 2-year-old loggerhead sea turtles at the NOAA Fisheries Sea Turtle Facility (NOAA-STF) in Galveston, Texas, U.S.A. during a 3-week period in September – October 2004. Loggerhead turtles at this facility originate from hatchlings collected from Florida nesting beaches and are captive reared until 3 years of age, at which time they are released off the Florida coast. Details of animal husbandry at the NOAA-STF are described by Higgins (2003). Briefly, yearling turtles were housed

individually in 76 cm diameter (45 cm depth) circular plastic containers with mesh flooring. Containers were suspended in rectangular fiberglass raceways (6.5 x 2.0 x 0.6 m) filled with 6435 L of seawater at 29 °C–30 °C. The raceways were drained and new seawater pumped in every other day. Ambient air temperature in the facility was regulated at 30 °C. Skylights in the facility roof exposed the turtles to a natural light photoperiod that varied with season. At the time of our experiments, local sunrise and sunset were at approximately 0630 and 1730, respectively. Fluorescent lights above the tanks were used to supplement natural sunlight and were on from 0730 to 1600. Turtles were fed a ration of two squid (approximately 150–200 grams) three times a week when experiments were not being conducted. The average mass of turtles used in this study was 8.35 ± 0.14 (S.E.M.) kg and average straight carapace length was 42.08 ± 0.23 (S.E.M.) cm.

## Experiment Tank

A fiberglass rectangular tank was used to assess the behavior and tracking abilities of juvenile loggerhead turtles presented with untreated squid bait and chemically modified squid bait under steady, semi-turbulent flow conditions. The working section of the tank consisted of a 0.9 **x** 0.9 m start chamber separated from a larger (2.1 **x** 0.9 m) main chamber by a gate attached to a pulley system (Fig. 1). The tank was filled to a depth of 26 cm with seawater at 29 °C. A series of three plastic honeycomb baffles (Specialized Metals, Coral Springs, FL) were located on the side of the main chamber opposite the start chamber and gate. During trials, seawater was pumped through these baffles at a volume flow rate of 275 $l \cdot min^{-1}$ and drained out of the tank through a drain grate on the floor of the start chamber. A Marsh McBirney electromagnetic flowmeter (Model 2000) was used to generate flow profiles of the tank under trial conditions. Flow speed was 3–5 $cm \cdot sec^{-1}$ in the central portion of the flume and tapered off to < 1 $cm \cdot sec^{-1}$ along the tank walls.

## Treatments

Seventeen turtles were randomly selected and assigned to either the 2-phenylethanol (2-PEA) or tiger shark skin extract (TIGER) treatment group. Each turtle in the 2-PEA ($N$ = 8) and TIGER ($N$ = 9) treatment groups was subjected to three trials; an untreated squid trial, a chemically modified squid trial, and a control trial. For the untreated squid trial, a nylon bag containing ~ 20–25 g of chopped squid was secured onto the side of the baffle facing into the main chamber. The nylon bag was positioned 12 cm below the water surface along the midline of the tank. For the chemically modified squid trial, a nylon bag filled with ~ 20–25 g chopped squid marinated overnight in either 0.1 M 2-phenylethanol (2-PEA treatment group) or skin secretions obtained from live, wild-caught tiger sharks (TIGER treatment group) was secured to the baffle. The third type of trial was a control trial in which the nylon bag was simply filled with more nylon and secured to the baffle (i.e., no squid, untreated or chemically modified, was presented to the turtle). We conducted the nylon-only control trials to see if flow cues alone could induce the same behaviors that we observed for chemical trials. Trial order was randomized for each treatment group.

All chemicals used for these experiments were approved by the NOAA Fisheries Sea Turtle Facility staff veterinarian.

## Trial Protocol and Analysis

Turtles were fasted for 36–44 hours prior to trials and all trials were conducted between 0800 and 1600. A trial was initiated by placing a turtle in the start chamber with the gate closed. The turtle was left in the start chamber for a 20-minute acclimation period in static water, and then seawater flow through the tank was initiated. Seawater flowed through the baffles and the nylon bag, effectively pushing a plume of chemical towards the start chamber. Two minutes after flow began, the gate separating the start chamber from the main chamber was lifted, and the turtle was free to explore both chambers for 10 minutes. All trials were conducted in complete darkness. During the exploration period, the turtle's behavior was monitored and recorded using a system of infrared (IR) spotlights and IR-sensitive cameras interfaced with a digital video recorder (DVR) (A-1 Services Unlimited Inc., Bradenton, Florida).

We were primarily interested in whether or not turtles could use a combination of flow and chemical cues to successfully locate squid bait in the absence of visual cues, and if chemical manipulation of squid bait would reduce the turtles' ability and/or willingness to track and locate bait. Trial videos were analyzed to assess successful location of bait, as indicated by the turtle striking and attempting to consume the nylon secured to the baffle, and the length of time necessary for turtles to locate bait. The amount of trial time spent in the main chamber where the chemical plume originated was also recorded.

During trials, turtles frequently displayed a behavior in which they suddenly stopped swimming, put their nostrils to the tank floor, raised their front flippers to the side of their head, and used rear flippers to paddle backwards or spin in circles around the same spot. This searching behavior is typical of captive turtles feeding in tanks, and we refer to it as "backup" behavior. The frequency with which backups were displayed during trials was recorded.

Within each treatment group, a logistic regression for binomial data (S-Plus, Insightful Corporation, Seattle, WA) was used to analyze differences in the number of turtles that located bait between control, untreated squid, and chemically modified squid trials. If data met assumptions for parametric tests, we used one-way repeated measures analysis of variance between groups (ANOVA) to assess differences between trials in backup frequency and the amount of time spent in the main chamber of the tank where the chemical plume originated. If data did not meet assumptions of normal distribution and equal variance, then a non-parametric repeated measures ANOVA on ranks (Friedman rank test) was used to look for statistical differences between trials.

## RESULTS

Turtles showed limited ability to locate squid bait in complete darkness under the flow conditions created in our experiment tank (Fig. 2). During trials in which untreated squid was presented to turtles, only 25–33% of turtles successfully located squid within the 10-minute trial period for the 2-PEA and TIGER treatment groups. Logistic regression showed that chemical modification of squid did not alter the ability of turtles to find bait: there was no significant difference in the number of turtles that found bait in untreated squid, chemically modified squid, and control trials for the 2-PEA ($X^2 = 3.632$, df = 2, $P = 0.163$) or TIGER ($X^2 = 4.270$, df = 2, $P = 0.118$) treatment groups.

Because so few turtles actually located the bait, we could not make any meaningful statistical comparison of the length of time necessary for turtles to locate bait during squid, chemically modified squid, and control trials. Table 1 shows a summary of the time taken by turtles to locate bait for each trial type in the 2-PEA and TIGER treatment groups. No consistent trend was obvious. For the 2-PEA treatment group, turtles found chemically modified squid bait 2–3 times faster than they found untreated squid bait. In the TIGER treatment group, only one turtle successfully located the chemically modified squid bait, and its recorded time was about twice as long as the average time taken for turtles to locate bait in the untreated squid and control trials.

Despite their limited ability to actually locate squid bait using chemical cues, turtles showed alterations in behavior when squid was present in the experiment tank. In the TIGER treatment group, turtles displayed characteristic feeding behavior (i.e., "backup" behavior) with significantly greater frequency during untreated squid and chemically modified squid trials compared with control trials ($X^2 = 12.514$, df = 2, $P < 0.001$) (Fig. 3). There was, however, no difference in backup frequency between untreated squid and chemically modified squid trials, so treating squid bait with extracts from a natural predator (tiger shark) did not alter feeding behavior in captive loggerhead turtles. Likewise, we found no significant difference in backup frequency between untreated squid, chemically modified squid, and control trials for the 2-PEA treatment group ($F = 2.063$, df = 2, $P = 0.164$) (Fig. 3).

There was no significant difference in the amount of time that turtles spent in the section of the tank where the chemical plume was generated (i.e., the main chamber) between trials in the 2-PEA ($F = 1.951$, df = 2, $P = 0.179$) or TIGER ($F = 0.346$, df = 2, $P = 0.713$) treatment groups (Fig. 4).

## DISCUSSION

Given the difficulties of studying the oceanic stage of juvenile loggerhead turtles in their pelagic environment, our understanding of their foraging behavior and how their foraging behavior might lead them to interact with fishing gear is rather limited. A recent

extensive survey of gut contents of loggerhead turtles captured in the North Pacific high-seas driftnet fishery showed that a variety of prey items are consumed, with surface-dwelling pelagic snails, crabs, jellyfish, and tunicates comprising the majority of gut contents (Parker et al., 2005). Non-neustonic prey items that occur at depths up to 100 m are also represented in gut contents but are less common. Patchy prey distribution in the oceanic habitat likely fosters opportunistic feeding behavior in juvenile loggerhead turtles (Tomas et al., 2001; Parker et al., 2005), and it is hard to imagine that turtles would pass up a "free meal" of longline squid bait should they come across it in their oceanic wanderings. Both sea turtles and other large pelagic predators, such as the target fish species of commercial longline fisheries, are attracted to oceanographic features such as seamounts and convergent fronts where prey are concentrated (Polovina et al., 2000) increasing the chances that turtles will come into close proximity to fishing operations and making them susceptible to interactions with longline fishing gear. Indeed, satellite-tracking studies have demonstrated that in the North Pacific Ocean juvenile loggerhead turtles congregate at the Emperor Seamounts and along convergent fronts characterized by strong gradients in sea surface temperatures and chlorophyll—areas also exploited by longline fishing fleets (Polovina et al., 2000; Parker et al., 2003; Polovina et al., 2005).

The sensory cues used by juvenile sea turtles for orientation and prey-seeking in the open ocean are not fully understood. Association with oceanic fronts, where prey is more readily available, may be maintained by using temperature and current cues (Polovina et al., 2004). Loggerhead turtles are generally considered to be visual predators, but it is possible that chemical cues associated with high concentrations of prey items in frontal zones contribute to the turtles' ability to detect and locate food in the open ocean. Use of chemical and flow cues in open-ocean orientation and migration has been demonstrated in salmon (Doving, 1990; Doving and Stabell, 2003), but has yet to be demonstrated for marine turtles. Results from our laboratory study of chemical orientation in juvenile loggerhead turtles do not provide strong evidence that turtles are effective at using only chemical and flow cues to locate a food source. Flow conditions in our flume tank were necessarily different from what turtles experience in the pelagic environment; however, we attempted to create uncomplicated flow conditions that would be conducive for tracking a turtle's response to prey treatments, i.e., a unidirectional current of low-to-moderate speed with a food source located upstream. Only 20–33% of turtles found squid bait presented under these simple flow conditions in the absence of visual cues. Flow conditions in the loggerhead turtle's pelagic habitat are undoubtedly more complex and dynamic than those we could create in the laboratory. The ability of turtles to use additional cues provided by currents and oceanographic gradients to orient towards a source of attractant chemicals in the oceanic environment is unknown and warrants further investigation.

Constantino and Salmon (2003) reported that post-hatchling leatherback turtles showed a rheotactic response when food homogenate was introduced into a test chamber through an underwater filter outflow, but we observed no strong rheotactic response to the current created in our flume tank. Behavior during trials was characterized by turtles moving back and forth between the start chamber and the main chamber along the sides of the tank, with occasional swims down the center of the tank, both facing into and away from the current. We found that the presence of food chemicals resulted in an increase in backup

behavior, wherein turtles searched the tank floor for the source of food odors, rather than a rheotactic response in which turtles searched "upstream". Backup behavior is an artifact of captive rearing; the tank floor is the most likely place loggerhead turtles will encounter food in their holding tanks. This obviously is not true for wild loggerhead turtles in the open ocean. Further experiments with turtles acclimatized to feeding under more natural flow conditions may help clarify the role of flow cues in feeding behavior. In addition to chemical and flow cues, tactile cues may also play a role in bait striking behavior of loggerhead turtles. Five of the nine turtles in the TIGER treatment group bit the nylon bait bag even when there was no squid inside (Fig. 2).

Although chemical cues elicit feeding behavior in loggerhead, green, and leatherback turtles (Grassman and Owens, 1982; Owens et al., 1982; Steele et al., 1989; Constantino and Salmon, 2003), most experimental evidence suggests that visual cues are of primary importance in foraging success. Constantino and Salmon (2003) found that when visual and chemical cues associated with jellyfish, a primary prey of leatherback turtles, were simultaneously presented to leatherback post-hatchlings, the turtles ignored the current created by chemical delivery and oriented towards the visual stimuli instead. When tested separately, visual stimuli evoked a more robust feeding response than chemical stimuli (Constantino and Salmon, 2003). Our experiments support the idea that sea turtles are primarily visual predators, as juvenile loggerhead turtles showed a low success rate locating food in the absence of visual cues (Fig. 2). For this reason, it seems likely that use of a visual deterrent, rather than a chemical deterrent, would be a more effective means of preventing sea turtle interactions with longline gear. Researchers with the NOAA Sensory Biology Working Group are currently investigating visual capabilities of sea turtles and pelagic fishes in an attempt to identify visual attractants and repellents (Fritsches et al., 2005; Wang et al., 2005). Even if an effective visual deterrent is identified and implemented in longline fisheries, however, bait chemicals in the vicinity of fishing operations may alert turtles to the presence of food and induce a heightened awareness of prey leading to alteration of searching behavior. The effectiveness of a visual deterrent will depend largely on whether or not the turtle's aversion response overrides the heightened feeding response, which is fueled in part by chemical cues. Studies investigating the efficacy of various methods for repelling birds show that a combination of both visual and chemical deterrents is more effective than either on its own (Mason and Clark, 1996).

Unfortunately, an effective chemical deterrent has yet to be identified for sea turtles. Previous studies have shown that loggerhead turtles readily consumed squid that had been soaked in lactic acid, urea, quinine hydrochloride, capsaicin, wasabi oil, and natural toxins (ink from *Aplysia* spp.) (J.B. Swimmer, personal communication). One approach we took for the current study was to assess the feasibility of disguising the odor of longline bait with a novel chemical, such as 2-phenylethanol, as a means to prevent loggerhead turtles from biting squid bait. Treatment of squid bait with 2-phenylethanol did not significantly alter the behavior of loggerhead turtles during trials, so the odor-masking approach using this particular chemical was ineffectual. Although wash-off in the current could have decreased the concentration of 2-phenylethanol over the course of the 10-minute trial, it is unlikely that this affected the results, as turtles located and bit squid bait marinated in 2-phenylethanol in less time that it took them to locate untreated squid bait (Table 1).

We were also interested in assessing the behavior of loggerhead turtles in response to chemical compounds derived from sharks, the main natural predators of juvenile and adult sea turtles. Higgins et al. (2005) demonstrated that captive-reared juvenile loggerhead turtles in nearshore holding pens show defensive behavior upon encountering a shark-shaped decoy and subsequently avoid the section of the pen where the decoy is present, providing experimental evidence that visual recognition plays a strong role in predator avoidance. The role of chemical recognition in predator avoidance has not previously been investigated for sea turtles. Terrestrial reptiles and amphibians display avoidance and defensive behavior when presented with skin extracts and rinses from predatory snake species or when placed in environments that have been "conditioned" by predator presence (Dial, 1990; Weldon, 1990); however, we found no significant difference in behavior of loggerhead turtles between untreated squid trials and trials in which squid had been treated with skin secretions from live wild-caught tiger sharks. If association of a predator's scent with a threat is learned rather than innate, then this may explain why shark-derived chemicals did not alter the behavior of captive-reared loggerhead turtles -- with no previous exposure to tiger shark scent -- during bait-tracking trials. Behavioral responses to predator-derived chemicals may be more pronounced in wild-caught sea turtles.

## CONCLUSIONS

In conclusion, we found that although loggerhead turtles detect and respond behaviorally to the presence of food chemicals in their aquatic environment, they have limited success in tracking and locating a food source using only chemical and flow cues. Visual cues are likely of primary importance to foraging success in loggerheads, with the chemical senses playing a secondary role. Further research is necessary to identify sensory deterrents and evaluate the feasibility of using sensory deterrents to reduce or prevent sea turtle bycatch in longline fisheries.

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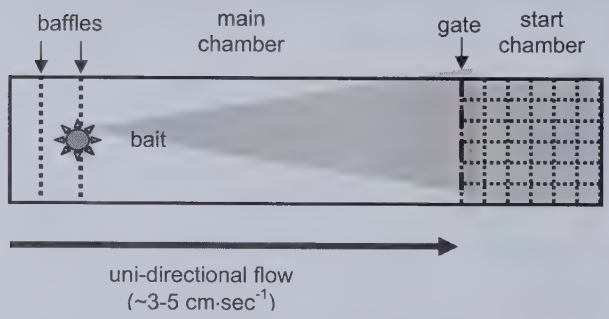
Table 1.--Length of time necessary for loggerhead turtles to locate and strike untreated squid, chemically-modified squid, and control bait in the 2-phenylethanol (2-PEA) and tiger shark extract (TIGER) treatment groups.

| **TREATMENT** *& trial type* | Time to locate bait (min)* | Number of turtles that located bait |
|---|---|---|
| **2-PEA** | | |
| *squid* | 2.57 ± 0.94 | 2 |
| *2-PEA* | 7.48 ± 0.02 | 2 |
| *control* | n/a | 0 |
| **TIGER** | | |
| *squid* | 4.71 ± 1.00 | 3 |
| *tiger* | 9.83 | 1 |
| *control* | 5.80 ± 1.27 | 5 |

*Results presented as arithmetic mean ± standard error of the mean (S.E.M).

Figure 1.--Diagram of tank used in experiments to assess chemical tracking abilities and behavior of 2-year-old loggerhead turtles at the NOAA Fisheries Sea Turtle Facility in Galveston, Texas.



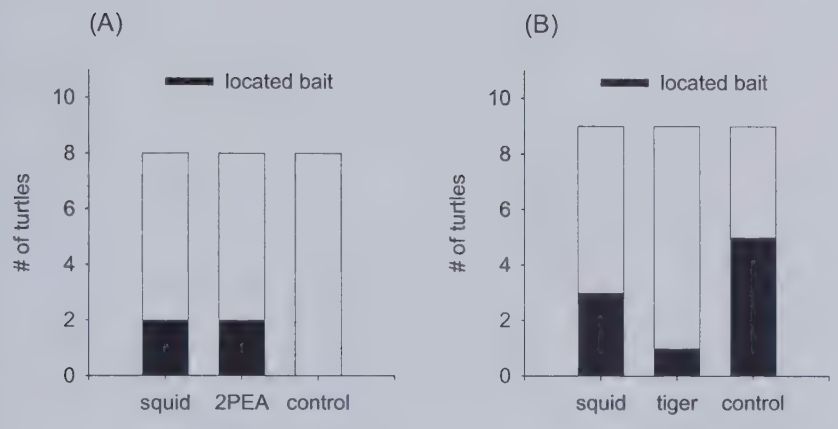



Figure 2.--Number of 2-year-old loggerhead turtles that successfully located bait during untreated squid, chemically modified squid, and control trials for the (A) 2-PEA and (B) TIGER treatment groups. White bars represent the total number of turtles used in trials (*N*). Black bars represent the number of those turtles that found bait.

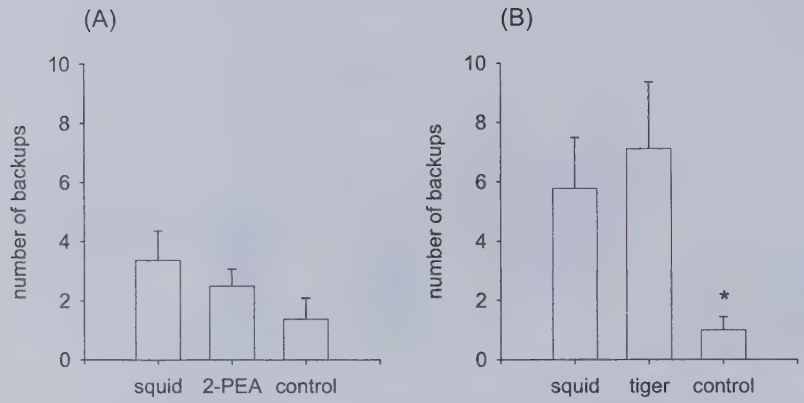



Figure 3.--Frequency with which backup behavior was exhibited by loggerhead turtles presented with untreated squid, chemically modified squid, and control bait for the (A) 2-PEA ($N = 8$) and (B) TIGER ($N = 9$) treatment groups. Turtles in the TIGER treatment group displayed backup behavior significantly more often during untreated squid and chemically modified squid trails compared with control trials ($X^2 = 12.514$, df = 2, $P < 0.001$).

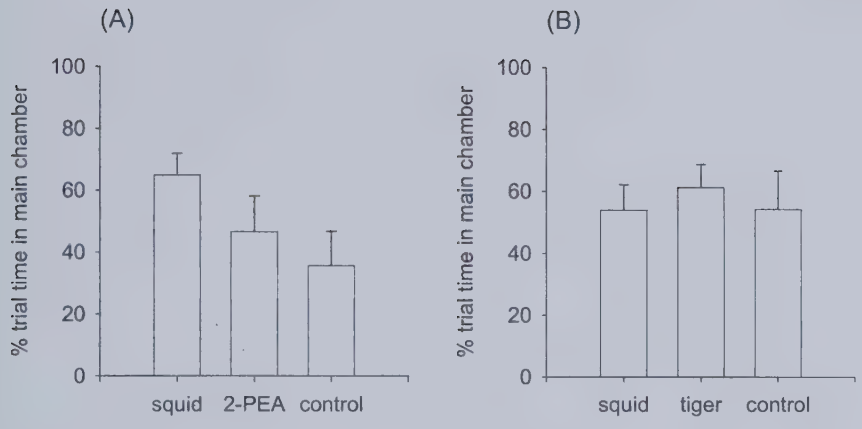



Figure 4.--Amount of time turtles spent in the main chamber of the experiment tank, where the chemical plume originated, during untreated squid, chemically modified squid and control trials for (A) 2-PEA ($N = 8$) and (B) TIGER ($N = 9$) treatment groups. In both treatment groups, there was no significant difference in time spent in the main chamber between the three trial types.

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